ON THE TRANSMISSION OF HUMAN TRYPANOSOMES BY *GLOSSINA MORSITANS*, WESTW.; AND ON THE OCCURRENCE OF HUMAN TRYPANOSOMES IN GAME

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I. INTRODUCTION

The experiments detailed below, on the transmission of human trypanosomes by means of *Glossina morsitans*, Westw., have been carried out at Nawalia, N. Rhodesia, situated approximately 12° 25′ S., and 32° 2′ E., on the right bank of the Nyamadzi River, a tributary of the Luangwa. In a direct line, the laboratory is about fourteen miles west of the Luangwa River, and lies at a height of about 150 feet above the level of that stream, which is, in this locality, roughly 1,900 feet above the sea.

Owing to various unforeseen circumstances, including a delay in

the receipt of equipment, it was impossible to commence work until the middle of June, 1911, so that during the greater portion of the time covered by the experiments the meteorological conditions have been those of the dry season, notably a high mean temperature, combined with a very low percentage of relative humidity. These conditions obtained until the end of November, when the rains broke.

A further delay in the completion of our results has been caused by the fact that during the hotter months the breeding flies died comparatively rapidly, and produced, also, a large percentage of abortions.

II. STRAIN OF HUMAN TRYPANOSOMES EMPLOYED

Quite recently, Stephens and Fantham (1910) have described some peculiar morphological features which they observed in a strain of trypanosomes derived from the Luangwa Valley, the chief of which consisted in a markedly posterior displacement of the macronucleus.

The animal reactions of the same strain, worked out by Bevan (1911), Yorke (1910), and others, have shown it to be particularly virulent for all the animals employed, and accordingly, on morphological and pathogenic grounds, the species *Trypanosoma*

* In Stephens' and Fantham's original paper they write: 'It may be stated at once that the peculiarity of this Rhodesian trypanosome is that among the short or stumpy forms some have the *nucleus at the posterior* (*non-flagellar*) *end* . . . . . the position of this posterior nucleus varies. Starting from the stumpy forms in which the nucleus is in the middle, we have all transitions up to that in which the nucleus is actually terminal and posterior to the blepharoplast.' While in the Transactions of the Society of Tropical Medicine and Hygiene for November, 1911, they say: 'He quotes Bevan as stating "that he has noted that in short forms the macronucleus may be situated slightly posterior to the centre of the parasite" . . . With regard to this . . . . neither we nor anybody else would attach importance to this fact alone . . . . the nucleus may actually lie on the posterior side of the blepharoplast. . . . . This we regard as the essential characteristic.'

If this second statement is intended to limit the meaning to be attached to the term 'posterior nucleus,' it appears to us that some confusion may result. The meaning which has been attached by one of us (W.Y.) to the term in other papers is that conveyed in Stephens and Fantham's original paper, and that is our interpretation in the present communication. Forms in which the macronucleus is actually posterior to the blepharoplast are of extremely rare occurrence, in our experience. On the other hand, those forms in which the macronucleus is displaced within the posterior quarter or fifth of the body of the trypanosome so that it comes to lie in contiguity to the blepharoplast, are of very common occurrence, often forming an appreciable percentage of the parasites found in an animal's blood on certain days. This displacement constitutes, in our opinion, the chief morphological peculiarity of this strain. Such forms have not been described, as yet, in any of the mammalian trypanosomes other than the human strains obtained from the Luangwa Valley and Nyasaland. We except, of course, *Trypanosoma transvaaliense*, which could give rise to no confusion.

rhodesiense was created by Stephens and Fantham (1910). A similar parasite has been described from a case of human trypanosomiasis originating in Nyasaland by Stannus and Yorke (1911).

The exact relationship which this trypanosome bears to *Trypanosoma gambiense* is still an open question, and without discussing the validity of the species, we desire at present simply to record the fact that we have inoculated rats from twelve cases of human trypanosomiasis, eleven of which were isolated from villages in the Luangwa Valley, for the most part situated on the main roads, and in every instance have observed the peculiar displacement of the nucleus already mentioned. The animal reactions of such of these strains, on which work has been done, agree with those of the Armstrong strain, as described by Yorke (1910).

It will be understood, therefore, that in our transmission experiments the strains of human trypanosomes utilised answer in all respects to the description of *T. rhodesiense*.

III. TRANSMISSION OF THE TRYPANOSOME

A. By Laboratory-bred *Glossina morsitans*

Certain general conditions which attach to all the experiments may be mentioned.

The identity of the flies has been controlled both by direct examination of the external characters, and by the preparation of the male genitalia, as recommended by Newstead (1911), so that we can state with some degree of confidence that we have been dealing only with *Glossina morsitans*, Westw.

All the experimental animals have been kept in fly-proof cages, the fronts of which were protected by a double layer of wire gauze, the inner composed of coarse, and the outer of mosquito meshing. The two layers were separated by a space of one inch in order to obviate the possibility of an animal being bitten while pressing its body against the front of the cage.

The feeding of the flies, and the changing into fresh bottles daily was done personally by one of us. The flies were kept in such a manner that they had no opportunity of obtaining food from other than the animals used in the actual experiments. Each fly was

preserved in a separate bottle, and had a special number, so that an exact history of its life, the number of meals it had, the animals on which it fed, and other particulars, were available.

Experiment 1. Commenced August 20th, 1911.

It is somewhat difficult to tabulate this experiment, owing to the fact that it was not started with a definite number of flies. Between August 20th and September 29th, twenty-six flies had hatched out, and each, as it did so, was given its first meal on an animal showing numerous parasites in the peripheral blood, so that on any given date the periods which had elapsed since the infecting feeds of the flies varied considerably. In Table 1 the main facts in connection with the flies are given.

Twenty-four hours after each fly had fed on an infected animal, it was afforded an opportunity of feeding on a clean monkey (No. 41); after forty-eight hours on a second (No. 42); and from the third day onwards on a third (No. 52). The schedule of feedings is given in Table 2.

From this table it will be seen that neither of the first two monkeys on which the flies were allowed to feed became infected, whereas No. 52 did so on the 27th of September.

A reference to Table 1 will show that up to, and including, the 26th of September, twenty-three flies had fed on an infected animal more than three days previously, and had, accordingly, been fed on Monkey No. 52. The three flies A 24, 25, and 26, had never fed on this animal, and therefore had not to be considered in the attempt to isolate the infected fly. Moreover, six of the flies, A 1, 3, 9, 11, 13, and 20, had died prior to the 26th of September, and of these, three proved to be negative throughout on examination. The other three, namely A 3, 11, and 20, were found to show a heavy intestinal infection of trypanosomes. Fly A 3 died on September 6th, A 11 on September 12th, and A 20 on September 13th, while the monkey did not become infected until September 27th, much too long an incubation period for one of these flies to have been the infecting one. We have additional proof for this conclusion in that the abdominal contents (gut + salivary glands) of flies A 3 and 20, on inoculation into monkeys, did not determine an infection.

On the 26th of September there were, then, twenty flies with which to deal, amongst which was at least one infected one. As

TABLE 1.—Giving date of infecting meal, date of death, and duration of life after infecting meal.

No. of fly	Date of infecting feed	Date on which fly died	Duration of life from date of infection
A 1	20/8/11	22/9/11	33 days
A 2	21/8/11	20/10/11	60 ,,
A 3	25/8/11	6/9/11	12 ,,
A 4	26/8/11	23/10/11	58 ,,
A 5	27/8/11	28/10/11	62 ,,
A 6	27/8/11	17/10/11	51 ,,
A 7	28/8/11	11/10/11	44 ,,
A 8	29/8/11	11/11/11	74 ,,
A 9	30/8/11	12/9/11	13 ,,
A 10	31/8/11	20/10/11	50 ,,
A 11	31/8/11	12/9/11	12 ,,
A 12	3/9/11	27/9/11	24 ,,
A 13	3/9/11	5/9/11	2 ,,
A 14	5/9/11	28/9/11	23 ,,
A 15	6/9/11	25/10/11	49 ,,
A 16	7/9/11	4/11/11	58 ,,
A 17	8/9/11	3/10/11	25 ,,
A 18	8/9/11	14/10/11	36 ,,
A 19	9/9/11	19/10/11	40 ,,
A 20	9/9/11	13/9/11	4 ,,
A 21	13/9/11	23/10/11	40 ,,
A 22	16/9/11	14/11/11	59 ,,
A 23	17/9/11	27/10/11	40 ,,
A 24	25/9/11	3/11/11	39 ,,
A 25	26/9/11	29/9/11	3 ,,
A 26	26/9/11	29/10/11	33 ,,

stated above, three flies, A 24, 25, and 26, had never fed on Monkey No. 52, so that the enquiry was limited to seventeen, and this was further reduced, by the death of flies A 12 and 14 on

TABLE 2.—Showing transmission of human trypanosomes by laboratory-bred *Glossina morsitans*.

Date	Animal	No. flies fed	Result	Remarks
Aug. 21-Sept. 18 ...	Monkey 41	5*	Negative	Flies fed 24 hours after infecting feed
.. 22 .. 28 ...	.. 42	14*	..	Flies fed 48 hours after infecting feed
.. 23 .. 26 ...	.. 52	23	Infection	Flies fed 72 hours and onwards after infecting feed
	.. 68	5	Negative	(*a*) Infecting feed over 30 days before
Sept. 27 and 28	.. 69	6	..	(*b*) Infecting feed between 20 to 30 days before
	.. 70	5	Infection	(*c*) Infecting feed less than 20 days before
Oct. 4	.. 72	16	..	All the flies fed
.. 4	White rat 77	16	..	All the flies fed
.. 5	Monkey 68	14	Negative	Infected fly did not feed
.. 6-9	.. 58	16	Infection	All the flies fed
.. 9	.. 61	1	,,	Infected fly, only, fed
.. 11-12 ...	.. 68	14	..	Infected fly fed on 13th and 14th as well
.. 13-16 ...	.. 69	13	Negative	Infected fly did not feed
.. 16-19 ...	.. 83	12	Infection	Infected fly commenced feeding on 16th, others on 17th
.. 25-28 ..	.. 69	10	Negative	Infected fly did not feed
.. 29-Nov. 11 ...	.. 56	4	..	Infected fly did not feed

* The remaining flies refused to feed.

September 27th and 28th—both flies negative on examination—to fifteen. These were accordingly split up into three groups, based on the length of time which had elapsed since the date of the

infecting feed, and each group was allowed to feed for two days on a clean monkey, Nos. 68, 69, and 70.

Group (*a*). Infecting meal over thirty days previously.

Group (*b*). Infecting meal between twenty and thirty days previously.

Group (*c*). Infecting meal less than twenty days previously.

Of the three monkeys, No. 70 was the only one to become infected, and the transmitting fly was thus located in Group (*c*), consisting of A 19, 21, 22, and 23.

While waiting to ascertain which of the three monkeys would become infected, all the flies were fed from September 29th to October 3rd on Monkey No. 72, and on October 4th on White Rat No. 77. Both of these animals became infected in due course.

On October 5th all the flies, with the exception of A 19, were re-fed on Monkey No. 68, and from the 6th to the 9th all were fed on Monkey No. 58, except on October 9th, when Fly A 19 alone was fed on Monkey No. 61. Of these animals, No. 68 did not become infected, while Nos. 58 and 61 did. The Fly A 19 (♂) was thus proved to be the infecting one.

No particular interest attaches to the further experiments. From the Table it will be seen that all those animals on which Fly A 19 fed became infected (Nos. 68 and 83), whereas those on which it did not feed remained quite healthy (Nos. 69 and 56).

When it had been definitely ascertained which was the infecting fly, it was possible to determine fairly accurately the duration of the cycle of the trypanosomes in the insect. Fly A 19 had its infecting meal on September 9th, and Monkey No. 52, the first to become infected, showed trypanosomes in the peripheral blood on September 27th. On the 26th, therefore, the last day on which the flies were fed on this animal, a period of eighteen days had elapsed since A 19 fed on the infected animal. The average incubation period of the local strain of human trypanosomes in monkeys is four or five days, and subtracting this from the eighteen days, it is evident that the fly must have become infective in thirteen days.

This fly, A 19, lived for forty days from the date of the infecting meal, and between the time of becoming capable of transmitting the parasite and the date of death, had fed on eight animals, all of which became infected.

The other flies were fed continuously from the date of the possible infecting meal to that of death, which occurred at varying periods from two to seventy-four days, but none of them became infective.

Experiment 2. Commenced November 14th, 1911, with sixteen laboratory-bred *Glossina morsitans*.

In this experiment the flies were infected directly on a case of Human Trypanosomiasis, each fly being allowed to feed on one occasion only. Ten fed on the 14th of November, when the patient showed three trypanosomes per field in the blood, and the remaining six on the 15th, when there was, on an average, one trypanosome to seven fields (Zeiss Oc. 4, Obj. DD.).

The subsequent meals are shown in Table 3.

TABLE 3.—Showing transmission of human trypanosome by laboratory-bred *Glossina morsitans*.

Days after infecting feed	Animal	No. flies fed	Result	Remarks
1st to 5th ...	White rat 116	15	Negative	
6th to 10th ...	,, 118	15	,,	
11th to 15th ...	,, 124	15	Infection	
16th to 20th ...	,, 116	13	,,	
21st to 22nd ...	,, 118	12	,,	
23rd to 27th ...	Monkey 137	6	Negative	Flies divided into two groups to separate the infective one
,, ,, ...	,, 138	5*	Infection	
28th to 42nd ...	,, 148	Varied	Negative	

* One fly of this group (6) refused to feed, and died on the 26th day of the experiment.

Rat No. 124 became infected on December 4th, five days after the flies had fed last, and as the incubation period of the trypanosome in these animals, on an average, is five days, it seems probable that the fifteenth day was the one on which the infecting fly became capable of transmitting the parasite.

On the 7th of December (twenty-third day after infecting meal), the twelve flies then alive were divided into two groups, in order to effect an isolation of the infective one, and were fed on Monkeys

Nos. 137 and 138, as indicated in the Table. On December 12th, the fly numbered B 23 (♂) died, and on examination proved to be heavily infected throughout the alimentary canal, and in the salivary glands. No infection of the proboscis, however, was observed.

The other flies were fed on a clean monkey until the forty-second day, but without any result.

B. By 'Wild' *Glossina morsitans*

Experiment 3. Commenced on November 14th, 1911, with ninety-eight 'wild' flies.

Prior to infecting these flies with the human trypanosome, they were fed for three days, November 14th to 16th, on a healthy monkey (No. 95), and for the next four days on a native fowl. The monkey never became infected. From the 21st to the 24th of November the insects then alive, fifty-seven in number, were fed on an infected monkey showing twenty to thirty trypanosomes per field in the peripheral blood, and were afterwards fed on healthy animals, as in Table 5.

Table 5.—Result of feeding 'wild' *Glossina morsitans* on clean monkeys, after a preliminary meal on an animal infected with the human trypanosome.

Days after *first* infecting feed	Animal	No. flies fed	Result	Remarks
4th to 6th ...	Monkey 125	48	—	Monkey died on 7th day
7th to 9th ...	,, 127	41	Negative	
10th to 13th ...	,, 130	34	Infection	
14th to 16th ...	,, 119	31	,,	
17th to 18th ...	,, 140	7	—	Monkey died on 19th day
17th to 23rd ...	,, 141	10	Infection	Flies divided into two groups to isolate infective ones
20th to 25th ...	,, 144	4	,,	

This experiment was finished after the flies had fed on the twenty-fifth day, the flies being then killed and embedded.

The duration of the cycle of the parasites in the flies, in this instance, would appear to be slightly over eleven days. The first

infecting meal was taken on November 21st, and Monkey No. 130 showed parasites in the peripheral blood on December 7th, a difference of sixteen days. As stated already, the incubation period of this trypanosome in monkeys is about five days, and by subtracting this from the sixteen days, we obtain eleven for the duration of the cycle.

IV. TRANSMISSION OF THE HUMAN TRYPANOSOME BY *GLOSSINA MORSITANS*, IN NATURE

The following experiments were two of a series undertaken to ascertain what varieties of trypanosomes were being transmitted by *Glossina morsitans*, in Nature. In these, varying numbers of freshly-caught flies were fed on monkeys immediately on reaching the laboratory, and while, in several cases, it was found that monkeys were successfully infected by the bites of such flies, these two experiments were the only ones in which the parasite concerned corresponded to the human organism.

Experiment 4. Commenced October 30th, 1911, with sixty freshly-caught flies, to which were added twenty-two additional ones on the next day. The flies were fed as indicated in Table 6.

TABLE 6.—Showing the transmission of the human trypanosome by naturally-infected *Glossina morsitans*.

Date	Animal	No. flies fed	Result	Remarks
Oct. 30-Nov. 4 ...	Monkey 96	60+22	Infection	
Nov. 6	,, 105	29	,,	
Nov. 7-10	,, 108	19	,,	
Nov. 11-12 ...	,, 113	7	Negative	Flies divided into two groups to isolate the infected fly.
,, ,, ...	,, 114	6	Infection	

On November 13th, the thirteen flies still alive, were killed and embedded. In the sections, numerous parasites were found in the gut and salivary gland of only one of them.

Experiment 5. Commenced January 7th, 1912.

In this experiment, all the 'wild' *Glossina morsitans* brought into the laboratory from day to day were fed on the one monkey, No. 210. This animal became infected on January 16th, and, allowing four days only as the incubation period of the parasite (the average, as stated earlier, is five days), must have been infected on the 11th or 12th. Including the 12th of January, 269 flies had fed on the monkey.

The conclusion that the trypanosome transmitted by the flies in these experiments is the human one, is based on (1) the morphology, and (2) the animal reactions.

(1) MORPHOLOGY.

This is identical with that of the strains we have isolated from infected natives, and with that of the strain described by Stephens and Fantham (1910). It shows the same marked dimorphism; short, non-flagellated forms, with the decided posterior displacement of the macronucleus, and the peculiar arrangement of the granules in the anterior portion of the body, viz., a row along either side of the trypanosome with a clear strip of protoplasm intervening; and long, free-flagellated forms, many of them of the prominent 'snout' type. The measurements, also, correspond with those of known human strains.

TABLE 7:—Comparison of the measurements* of the 'fly' trypanosome with those of strains obtained from cases of Sleeping Sickness.

Strain	No. measured	Length in microns		
		Average	Maximum	Minimum
Human ...	... 500	21.32	32.25	13.27
'Fly'	... 500	22.58	36.25	14.5

* The method we have adopted in measuring the trypanosomes is essentially that described by Bruce. The blood smears were dried in the air, fixed in absolute alcohol, and stained with Giemsa. Five hundred parasites, in each case, taken as they came, were drawn with the camera lucida at a magnification of 2000 diameters, and the length measured along the middle line. Only 25 were drawn from a preparation made on any one day, and dividing forms were not included.

The curves obtained by plotting out the distribution of the various lengths, expressed in percentages of the number measured, will be found in the chart on page 20.

(2) Animal reactions.

Table 8 gives a synopsis of the course of the disease in the animals.

Table 8.—Pathogenicity of the 'fly' trypanosome.

Animal	Incubation period in days	Duration in days
Monkey 96	6	47
,, 105	4	54
,, 108	4	9
,, 114	6	42
,, 210	4	—
Rat 103	3	24

The number of animals is very small, but is sufficient to demonstrate the virulence of the strain.

Discussion of the Results

In these transmission experiments, there are at least three sources of error which must be considered, (1) accidental infection of the experimental animals by other than the experimental flies, (2) hereditary transmission of trypanosomes from infected female flies to their progeny, and (3) natural infection in the experimental animals.

(1) With regard to the first of these, the conditions under which the experimental flies and animals were kept have been mentioned already, and it seems more than improbable that accidental infection would account for the unfailing regularity with which the animals became infected after the infective flies had fed on them. Moreover, in all our experiments to date, well over 200, such an occurrence as the unexpected infection of an animal has not been observed.

(2) The number of bred flies which we have been able to obtain has been too small to permit us to examine many of them prior to use in the experiments, but such as were, have been found uniformly free of infection. Stuhlmann (1907), Kleine (1909), and Bruce

(1911, *a*) with his colleagues, have examined large numbers of bred flies belonging to the species *Glossina brevipalpis*, *Glossina morsitans*, and *Glossina palpalis*, and are unanimous in the opinion that a hereditary transmission of trypanosomes does not occur amongst the tsetse flies.

(3) With reference to the third point, we have used in the course of our work well over 100 monkeys, and have never yet seen a naturally-occurring trypanosome infection in any of them. *Plasmodium kochi* has been observed occasionally, but beyond this, nothing. Experiment 2 was specially devised to obviate the possibility of error through the use of local monkeys (*Cercopithecus pygerythrus*). The flies, as they were obtained, were fed on healthy, imported rabbits, which showed no signs of infection throughout; they were infected directly from the human host; and they were then fed on white rats.

The genealogy of the strain used in Experiment 1 was as follows:—

Human Host

↓

Monkey No. 5

↓

Guinea-pig No. 14

The monkey, before inoculation, had been carefully watched for fifteen days, and during this time had showed no signs of an infection, while the monkeys Nos. 41, 42, and 52, on which the flies were afterwards fed, had been examined daily for a week before they were used in the experiment, and had thus been proved to be quite healthy.

The trypanosomes transmitted by these bred flies were identical with the human one, both morphologically and in their animal reactions.

There are certain points in connection with the experiments which appear to be worthy of emphasis. The number of bred flies which has been used in each is strikingly small, very much more so than in any other similar work of which the records are available. In the two experiments a total of forty-two was employed, and of

these two only became infective, a percentage of 4·76, or, considering only the number (twenty and fifteen) which lived over the duration of the cycle of the parasites in the insects, a percentage of 5·71.

With regard to Experiment 3, in which wild flies were used, no definite statement can be made as to the percentage infected, other than at least two were transmitting the trypanosome out of a total of fifty-seven. Freshly-caught flies have been shown to be transmitting other species of trypanosomes, and the finding of an intestinal or salivary gland infection in a fly which had not actually been proved to be transmitting the human parasite, would therefore be of no particular significance.

The time occupied by the trypanosomes in completing their cycle in the flies is also strikingly short, approximately two weeks (thirteen, fifteen, and eleven days). That of *Trypanosoma gambiense* in *Glossina palpalis* is ordinarily over twenty-one days, and in Taute's (1911) recent work with *Glossina morsitans*, the same time was taken by the trypanosome with which he was working. Kleine's (1909) experiments with the same species of *Glossina* were without result, though a very large number was used, and the experiments continued over a long period of time.

How to account for these different results is a problem which immediately presents itself. Are they due to different meteorological conditions? Or are they due to the use of different strains, or species, of human trypanosomes? Kleine's experiments were made on the shores of the Victoria Nyanza, at an altitude of 3,700 feet; Taute's on Lake Tanganyika, the height of which is 2,680 feet; and ours at Nawalia, at an altitude of, roughly, 2,000 feet; while the results may be epitomised as failure, flies infective after twenty-one days, flies infective after eleven to fifteen days. In this connection, we hope to have an opportunity of repeating the transmission work on the Congo-Zambesi watershed, which varies between 4,000-5,000 feet above sea-level.

So far as our results go, we have seen no indication of late infection in any of our flies, although some of them have lived as long as seventy-four days after the potentially infecting meal.

All our results go to show that mechanical transmission of the trypanosomes does not occur, that is, if a period of twenty-four hours has elapsed since the infecting meal. We have not made any

experiments to learn whether infection could be accomplished by interrupted feeding. This has been proved with various insects, but practically would account for very few, if any, cases of the disease.

The infective flies have been found to retain the power of transmitting the parasites during life, and do not require to feed more than a single time on any animal in order to infect it, neither do they require, prior to becoming infected, to feed more than once on an animal suffering from trypanosomiasis.

Our investigations on the manner in which an infective fly transmits the disease are incomplete, but seem to indicate that the explanation advanced by Bruce is the correct one. The infective flies have been found, both by direct examination of the various organs, and by cutting sections of the whole abdomen, to harbour trypanosomes in the gut and salivary glands. Three flies in Experiment 1, which died four, twelve, and twelve days after the infecting meal, were found to contain parasites in the gut, but the abdominal contents (including the salivary glands) of two of these, dead after four and twelve days, when injected into monkeys, did not result in infection. On the other hand, a portion of the anterior gut only of an infective fly, containing many trypanosomes, determined an infection in a white rat six days after inoculation. This would tend to corroborate Bruce's statement that the flies are non-infective until the cycle of the parasites has been completed. With the exception of the three flies mentioned, in addition to the permanently infected ones, all the others, dissected as they died, have been uniformly free of infection in the gut, salivary glands, and proboscis. An infection of the proboscis has never been observed in any of the bred flies which were transmitting the trypanosome.

Experiments 4 and 5 are of particular interest in that they dispose of the criticism that the successful transmission of the trypanosome in the laboratory does not necessarily mean that it occurs in nature. In all, 1,340 freshly-caught *Glossina morsitans* have been fed on nine monkeys, and while infection resulted in several, only two groups proved to be transmitting the human organism.

V. OCCURRENCE OF THE TRYPANOSOME IN GAME

The possibility that game might act as a reservoir of infection in Sleeping Sickness areas has been recognised almost since the inception of work on the disease, but up to the present it would appear that the trypanosomes have never been demonstrated in such animals under natural conditions. In Uganda, Bruce, Hamerton and Bateman (1911) have proved that certain species of buck, notably waterbuck, bushbuck, and reedbuck, can readily be infected with *Trypanosoma gambiense* by allowing infected *Glossina palpalis* to feed on them, and that healthy flies, in turn, may be infected from diseased game. They were unable, however, to examine a sufficiently large number of head to ascertain whether a natural infection was present.

The importance of the question is obvious, and the results of our investigations on the point afford a striking commentary on the potential danger involved in the infection of the game.

The Luangwa Valley is particularly rich in a widely-varied fauna, and owing to the fact that in the dry season the great bulk of the game tends to collect in the vicinity of the few permanent streams, it has been comparatively simple to shoot buck for the purposes of experimentation. The animals examined, and the findings, are given in Table 9.

It will be seen from this Table that ninety-eight animals, comprising nineteen genera, have been examined directly, and that inoculations have been made from fifty. The sub-inoculated animals have lived sufficiently long to enable us to determine their susceptibility to the various game trypanosomes.

In this vicinity there appear to be at least three well-differentiated trypanosomes affecting game: one which is closely allied to *Trypanosoma pecorum;* a second, to which monkeys and rodents are refractory, recalls, morphologically, *Trypanosoma vivax;* and a third which is identical, morphologically and in its animal reactions, with the human parasite isolated from local cases of the disease.

From the fact that *Trypanosoma vivax* has been found, it will be apparent that, by the use of monkeys and rats for the initial inoculations, a source of error in the exact estimation of the

percentage of game harbouring trypanosomes has been introduced. This has been unavoidable, owing to our inability to procure clean sheep or goats for the purpose. The percentage of game, 30·6,

TABLE 9.—Results of examination of game for trypanosomes.

Animal	Number examined	Number found infected by direct examination of blood	No. of inoculations	Number of positive inoculations in which parasites were found in peripheral blood	Number of positive inoculations in which no parasites were found in peripheral blood	Total number found infected by direct examination and by inoculation
1. Elephant (*Elephas africanus*) ...	1		1	0	0	0
2. Rhinoceros (*Rhinoceros bicornis*) ...	1	0	1	0	0	0
3. Hippopotamus (*Hippopotamus amphibius*)	1	0	0	0	0	0
4. Zebra (*Equus burchelli*)	3	0	2	0	0	0
5. Roan (*Hippotragus equinus*)	5	1	1		0	1
6. Wildebeest (*Connochaetes taurinus*) ...	2	0	1			-
7. Kudu (*Strepsiceros kudu*	7	3	3	1	1	4
8. Hartebeest (*Bubalis lichtensteini*) ...	2	0	1	0	1	1
9. Waterbuck (*Cobus ellipsiprymnus*) ...	26	15	14	5	1	16
10. Puku (*Cobus vardoni*)	1	1	6	1	0	1
11. Mpala (*Aepyceros melampus*) ...	18	1	11	1	1	2
12. Bushbuck (*Tragelaphus scriptus* ...	7	4	4	1	1	5
13. Bushpig (*Potomachaerus chaeropotamus*)	2	0	1	0	0	0
14. Warthog (*Phacochaerus aethiopicus*) ...	6	2	3	0	1	1
15. Lion (*Felis leo*)	2	0	0		0	0
16. Hunting dog (*Lycaeon pictus*) ...	1	0	1	0	0	0
17. Giant rat (?)	1	0	0	0	0	0
18. Genet (*Genetta rubiginosa*	2	0	0	0	0	0
19. Squirrel (?)	1	0	0	0	0	0
Totals	68	25	50	8	6	31

found to be infected with trypanosomes, with the means at our disposal, represents, therefore, the minimum for the country around Nawalia.

We reserve a discussion of the two varieties first mentioned for a future date, and shall deal here only with that one which appears to be the human parasite. Of the fifty buck from which inoculations were made, eight have proved to harbour this organism, a percentage of 16.

In Table 10 the animal reactions of the strains obtained from the various buck are given.

Table 10.—Pathogenicity of the *human* trypanosome from various species of game.

Animal	Subinoculations		Incubation period	Duration of infection
Waterbuck 1	Monkey	71	6 days	10 days
	,,	82	11 ,,	21 ,,
	,,	99	4 ,,	40 ,,
	,,	117	6 ,,	14 ,,
	Rabbit	79	4 ,,	3 ,,
	Guinea-pig	80	11 ,,	23 ,,
	Rat	81	3 ,,	21 ,,
Waterbuck 2	Monkey	170	5 ,,	8 ,,
	Rat	160	5 ,,	23 ,,
Waterbuck 3	Monkey	201	6 ,,	9 ,,
	Rat	178	6 ,,	
Waterbuck 4	Monkey	181	7 ,,	18 ,,
	Rat	213	5 ,,	
Hartebeest	Monkey	112	8 ,,	12 ,,
	,,	120	7 ,,	20 ,,
	,,	150	4 ,,	7 ,,
	,,	168	5 ,,	9 ,,
	Rat	128	4 ,,	46 ,,
Mpala 1	Monkey	169	5 ,,	9 ,,
	Rat	157	6 ,,	
Mpala 2	Monkey	199	4 ,,	
	Rat	176	5 ,,	
Warthog	Rat	195	5 ,,	
Native Dog	Monkey	131	5 ,,	11 ,,
	Rat	129	4 ,,	11 ,,

For the purpose of comparison, we give the incubation period and duration of infection in similar animals inoculated from known human strains.

TABLE 11.—Comparison of the pathogenicity of the *human* trypanosome from game with that of two strains obtained from cases of Sleeping Sickness.

Animal	Trypanosome from game		Local human trypanosome		'Armstrong' strain	
	Incubation days	Duration days	Incubation days	Duration days	Incubation days	Duration days
Monkey (14)	4-11	7-40	2-7	4-42	3-5	8-14
Rabbit (1)	4	30	4	16-61	3-14	19-45
Guinea-pig (1)	11	23	12-19	65-81	3-15	39-82
Rat (8)	3-6	11-46	2-8	15-43	1-7	6-45

The figures in parenthesis refer to number of each animal inoculated from game.

The extreme virulence of the strains derived from buck is most marked, and the correspondence between these reactions and those of known human strains is equally pronounced.

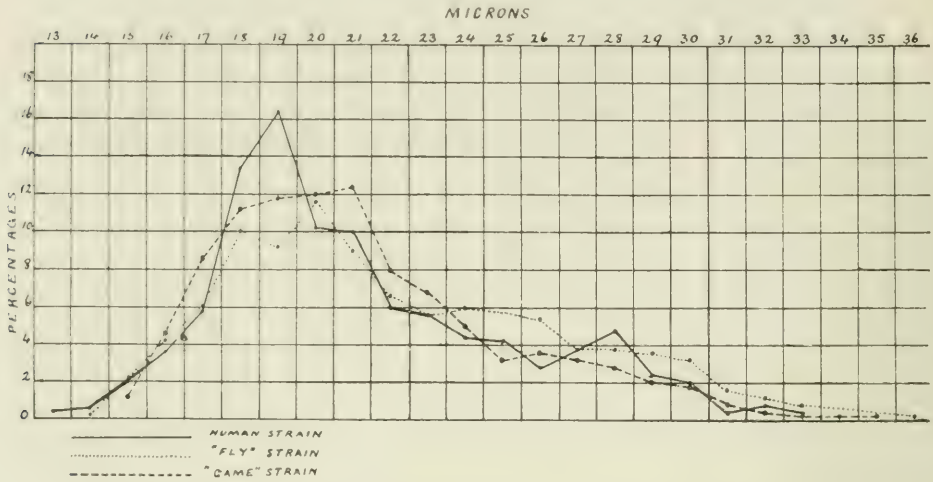
The measurements of the game strain of parasites are given in Table 12.

TABLE 12.—Comparison of measurements of *human* trypanosome from game with those of a known human strain.

Strain	No. measured	Length in microns		
		Average	Maximum	Minimum
Human	500	21.32	32.25	13.27
'Game'	500	21.35	35.5	15

The curves obtained by plotting out the distribution of the various lengths of the parasites, expressed in percentages of the total number measured, is given in the Chart for the human, 'game,' and 'fly' strains.

Chart, giving the percentages of the various lengths of the parasites encountered in an examination of 500 of each of the three strains, human, 'game,' and 'fly.'



The very striking resemblances in these curves is apparent at a glance, and a further similarity in the three strains of trypanosomes is evidenced by a comparison of the percentages of the 'stumpy and short,' 'intermediate,' and 'long' forms mentioned by Bruce (1911,[b]) in connection with *Trypanosoma brucei*.

Table 13.—Comparison of the percentages of 'short,' 'intermediate,' and 'long' forms of the human, 'game,' and 'fly' strains.

Strain	Short forms 13-21μ	Intermediate forms 22-24μ	Long forms 25-36μ
Human	62.4	16	21.6
'Game'	61.8	19.8	18.4
'Fly'	52.2	18.2	29.4

The curves of the strains which we have given above correspond more or less to that of *Trypanosoma brucei*, and it may be asked whether the parasites we have isolated from game, and from the animals infected by naturally-infected 'wild' flies, are not identical with this trypanosome. A comparison of the curves, and of the

percentages of the 'short,' 'intermediate,' and 'long' forms with those of *Trypanosoma brucei*, shows that there is a marked tendency for the trypanosomes to collect towards the 'short' and 'long' poles in the case of *Trypanosoma brucei*, whereas in our strains the bulk of the parasites are disposed at the 'short' pole.

TABLE 14.—Percentages of 'short,' 'intermediate,' and 'long' forms of two strains of *Trypanosoma brucei**.

Strain	Short forms 13-21μ	Intermediate forms 22-24μ	Long forms 25-36μ
T. brucei, Zululand ...	53.0	5.5	41.5
T. brucei, Uganda	39.3	21.8	38.9

* Compiled from tables in Reports of S.S. Comm. Roy. Soc., No. 11, 1911.

This distinction is, perhaps, rather a fine one, more particularly when one considers the comparatively small numbers of parasites from which the curves have been constructed.

We might state here that we are of the opinion that to be of any real value such curves must be plotted out from the measurements of a large number of trypanosomes, more especially when dealing with a dimorphic parasite of the human type, and we regret that time has not permitted us to measure a larger number than we have done. In any experimental animal infected with the human trypanosome, the proportion of short and long forms found in the blood on any one day is not equal; on one day practically nothing but short forms may be seen, while on another, only long forms may be present. It is obvious, therefore, that unless a limited number only of parasites be measured on any one day, and unless other preparations on later days be utilised, a source of error may be introduced, and false conclusions drawn.

Reference has already been made to the morphology of the human trypanosome in connection with that of the 'fly' strain. With regard to that of the 'game' strain, there is nothing to add except that it is identical with the other two, both in fresh and stained preparations. When the parasites are plentiful in

sub-inoculated animals, the short forms, with no free flagellum, exhibit the same posterior displacement of the nucleus, and the same arrangement of the granules, while among the long, free-flagellated forms, individuals of the 'snout' type are of frequent occurrence.

The presence, among the short forms, of parasites displaying the marked nuclear displacement, is confined, so far as our present knowledge extends, to the human strains originating in this part of Africa, and we must therefore conclude that these strains derived from the fly and game, exhibiting as they do the same morphology, measurements, and animal reactions, are identical with the local human trypanosome.

In addition to the game, the parasite was isolated from a native dog, found to be naturally-infected, in a village some fifty miles from the laboratory, where, so far as we are aware, the human disease has not been diagnosed.

VI. SUMMARY

1. The human trypanosome, in the Luangwa Valley, is transmitted by *Glossina morsitans*, Westw.

2. Approximately 5 % (4·76) of the flies may become permanently infected, and capable of transmitting the virus.

3. The period which elapses between the infecting feed of the flies and the date on which they become infective, is approximately fourteen days.

4. An infected fly retains the power of transmitting the disease during its life, and is infective at each meal.

5. Mechanical transmission does not occur if a period of twenty-four hours has elapsed since the infecting meal.

6. Some evidence exists to show that in the interval between the infecting feed and the date on which transmission becomes possible, the parasites found in the flies are non-infective.

7. *Glossina morsitans*, in nature, has been found to transmit the human trypanosome.

8. Certain species of buck, viz., waterbuck, hartebeest, mpala, and warthog, have been found to be infected with the human trypanosome.

9. A native dog has been found to be infected with the human trypanosome.

In conclusion, we desire to express our thanks to Dr. A. F. Wallace, M.O., N. Rhodesia, and to Mr. Ll. Lloyd, Entomologist to the Administration, for assistance during the course of the experiments.

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